IMMUNOHISTOCHEMICAL STAINING
PNA-HRP PROTOCOL

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TISSUE PREPARATION FOR SECTIONING

1. Label 15mL aluminum liquid N_2 containers (Accurate Chemical Scientific, 1-800-645-6264) containing 4mL of frozen tap H_20.
2. Sacrifice animals and drop the desired tissues in the aluminum container filled with liquid N_2. Keep groups separated. When liquid N_2 has evaporated, cap the container and store at –70°C.
3. To begin sectioning, mount the individual frozen tissue with tissue freezing medium (Scientific Products, 1-800-964-5227) and allow to freeze thoroughly.
4. Routine sections are trimmed at 20 μm and cut for slides at 5 to 8 μm and picked up on a labeled glass slide.

PNA-HRP STAIN

1. Remove frozen slides from the –70°C freezer and allow to warn to room temperature.
2. To contain the staining solutions, encircle the section including the tissue medium with a hydrophobic slide marker (PAP pen, Research Products International Corp., 1-800-323-9814).
3. Rinse 2-3X in 1X PBS.
4. Add 250μL of PNA-HRP (1:75 dilution in 1%BSA/PBS). (PNA-HRP, # L-7759, Sigma Chemical, 800.325.3010)
5. Rinse 2-3X in 1X PBS.
6. Add 250μL formamide to 4mg 3-Amino-9-Ethyl-Carbazole (AEC, Sigma: A-5754) and place in 9.75 mL sodium acetate buffer (.05M $CH_3COONa\text{-}3H_2O$, pH 5.0). Filter this solution (0.22mm) and add 50 μL of 3% hydrogen peroxide solution. Immediately add to the slides.
7. Developing takes 5-15 minutes and can be monitored under the microscope.
8. Rinse with 1X PBS 4 times for a minimum of 5 minutes each, longer aids in removing background.
9. To mount, apply a thin layer of crystal mount, being careful to NOT touch the tissue. Cover when drying to avoid dust.